

## Characterizing the Interaction Between Fire Ants (Hymenoptera: Formicidae) and Developing Soybean Plants

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**ABSTRACT** This research characterizes the interaction between the fire ants *Solenopsis invicta* Buren and developing soybean plants. Phagostimulant studies showed that fire ant foraging on soybean seeds increased once the seeds imbibed water. During seedling development over a 5-d germination period, fire ant foraging shifted from the stem/cotyledons to the roots, despite continual increases in fresh weights for each region, and the fact that stem/cotyledon tissue contained the majority of food reserves. Carbohydrate analysis showed that although 2-d-old seedlings had higher concentrations of phagostimulant carbohydrates, especially sucrose, than tissues of mature plants, all tissues analyzed had enough of these sugars to induce a phagostimulant response. Fire ant association with seeds/seedlings germinated in soil resulted in reduced seedling vigor, as determined by a doubling of seedling emergence time, a threefold increase in malformed seedlings, and visible damage to cotyledons. Seeds germinated and grown to mature plants in association with fire ants, allocated 43% more assimilate into pods, but produced 28% less root dry matter, 11% less total dry matter, and there was an 81% reduction in the number of root nodules compared with control plants. We propose that reduced root development and inhibitions of nodule formation would be major yield limiting factors under field conditions. This work demonstrates that fire ant damage to soybeans is not limited to seedling establishment and that more research should be directed at the subterranean activities of the fire ant.

**KEY WORDS** *Solenopsis invicta*, soybean, ant-plant interactions, fire ant, *Glycine max*

THE RED IMPORTED fire ant, *Solenopsis invicta* Buren, was accidentally introduced into the United States in the 1930s from South America. Since then it has spread to infest over 150 million hectares in 12 southern states, California, and Puerto Rico (Lofgren 1986). The fire ant exists in two distinct forms, monogyne (a single queen per nest) and polygyne (many queens per nest). The monogyne form reaches maximum carrying capacity at ≈100–120 mounds per hectare, with each colony containing ≈250,000 workers. Worker densities in polygyne populations are even greater with 300–2,000 smaller diffuse mounds per hectare (Porter et al. 1991). The energy requirement for this large biomass is enormous, and these large populations of predaceous fire ants have a significant impact on the ecology of the areas they inhabit. They have been shown to decimate native ants and other arthropods (Porter and Savignano 1990), as well as birds (Wilson and Silvy 1988) and mammals (Grant and Killion 1988).

The fire ant is agriculturally important because it is an opportunistic omnivore that has an excellent food recruitment system (Vander Meer 1986). The workers attack a wide variety of crops including soybeans, potatoes, corn, citrus, and okra; however, the nature of the interaction between crop plants and fire ants is

poorly understood. At least part of the crop damage in corn and soybeans is from direct feeding on germinating seeds (Adams 1986, Morrison et al. 1997). This kind of damage was noted soon after research began on the imported fire ants (Wilson and Eads 1949); however, the extensive use of persistent chlorinated hydrocarbon insecticides in the 1950s and 1960s subdued the agricultural effects of fire ants until these residual compounds dissipated in the 1980s to the present (Adams 1986). The resulting increase of fire ant related crop damage has generated a need for a better understanding of the interaction between fire ants and crop plants.

It is presumed that plants provide a significant portion of fire ant dietary carbohydrates. Carbohydrates are an important part of a fire ant diet. Studies in laboratory rearing of fire ants (Williams et al. 1980) demonstrated that honey-water added to the standard laboratory diet enhanced colony weight and queen survival. Also, several specific carbohydrates have been shown to be excellent fire ant phagostimulants (Ricks and Vinson 1970, Vander Meer et al. 1995). In addition, several food preference studies have also indicated the importance of dietary carbohydrates (Vinson 1968, Howard and Tschinkel 1981, Sorensen and Vinson 1981). Of the fire ant life stages, only fourth-instar larvae are capable of ingesting solid food (Glancey et al. 1981). Therefore, the energy requirements of workers and most life stages must be met

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through ingestion of liquids, and the portion of this that comes from plants should be rich in water-soluble compounds. Support for this comes from analysis of the crop contents of returning foraging workers that indicates carbohydrates, probably from plant roots or the exudate from root associated coccids, represented a large proportion of what was being brought back to the colony (Tennant and Porter 1991). It is estimated that at least 70% of the carbohydrates used to support colony maintenance and growth are obtained directly or indirectly from plants.

There are a number of ways in which fire ants could obtain plant-derived carbohydrates. The fire ant takes advantage of a variety of situations where carbohydrates and other food materials are available. This has been documented through observations of above ground fire ant-plant interactions, for instance feeding at extrafloral nectaries (Agnew et al. 1982, Vander Meer and Merdinger 1990, Lanza 1991) and homopteran tending (Kidd 1983, Banks et al. 1991). Underground fire ant feeding has also been studied using radioisotope labeled plants. Fire ant workers accumulated radioactive compounds when foraging in the vicinity of corn, okra, and soybeans injected with  $^{32}\text{P}$  (Smittle et al. 1983). In these experiments, fire ants were not observed feeding on soybean seedlings yet worker ants in the immediate vicinity had high levels of radioactivity. It was hypothesized that the ants were probably feeding on plant roots; however, the majority of the crop damage was assumed to be the result of damage during early seedling establishment that reduced the crop stand density (Adams et al. 1983).

The limited information available on the interaction of fire ants and commercially important crop plants does not yet provide a complete picture of the extent and nature of fire ant induced crop damage. Here we report on experiments designed to study the effect of fire ant association with soybeans during seed germination and plant development.

### Materials and Methods

**Plant Material.** Soybean seeds, *Glycine max* L., 'Kirby', harvested in autumn of 1993 were provided by Kuel Hinson (USDA-ARS, Agronomy Department, University of Florida, Gainesville, FL) and used for all experiments.

**Source of Fire Ant Colonies.** Laboratory colonies of *S. invicta* were either collected from monogyne field sites near Gainesville, FL, or were generated from newly mated queens also collected near Gainesville, FL. Colony rearing and maintenance procedures were based on standardized methods (Banks et al. 1981).

**Phagostimulant Effects of Soybean Seeds and Seedlings.** To determine if fire ants forage on freshly planted seeds, or feed on specific tissues of the seedling, experiments were performed to study the phagostimulant affects of seed and seedlings harvested at different developmental stages during germination and seedling growth. Five developmental stages of seed germination were defined as follows: (dry seed); (G-1) radicle emergence <1 cm; (G-2) radicle emer-

gence 1–2 cm; (G-3) radicle emergence (>2 cm and <5 cm) and cotyledon greening; (G-4) significant seed coat cracking, beginning of secondary roots, radicle  $\geq 5$  cm; (G-5) extensive secondary roots, seed coat beginning to fall off. To initiate germination, seeds were placed in rolls of moist germination paper and incubated in the dark at 25°C for the indicated length of time. Representatives from each of the defined germinating seed categories were weighed ( $n = 6$ ). The bioassay was similar to one already described for assessing fire ant carbohydrate phagostimulants (Vander Meer et al. 1995). Clean colony trays had numbered positions from 1 to 10 marked equidistant from each other and in a 15-cm radius from the tray center. The tray sides were painted with Fluon (ICI, Wilmington, DE) to prevent ant escape. Colonies starved for 24 h with two or more nest cells (10,000–20,000 workers each; Banks et al. 1981) were used for the bioassays. A colony nest cell and foraging workers were transferred to the center of a clean bioassay tray. Before testing worker ants were allowed to acclimate at least one hour.

Samples of treatments, controls, and standards were randomly placed around the 10 symmetrical locations on the tray floor. The bioassay was evaluated by counting the number of ants feeding at the plant parts or standard solution droplet every 5 min for a total of 30 min. The results for the six periods were added and the total used to calculate the ranking (a water control and 1% [wt:vol] sucrose standard was included in each test). The test samples were ranked by setting the water response at zero and the sucrose response at 100. This nullified much of the natural colony-to-colony variation and allowed comparison of results from one test to another. The ranking was calculated as follows:

$$\frac{\text{Response (sample)} - \text{Response (H}_2\text{O)}}{\text{Response (sucrose)} - \text{Response (H}_2\text{O)}} \times 100$$

The mean and standard deviation was calculated for each sample.

**Soybean Germination in Association with Fire Ant Colony.** Germination studies of soybeans in association with fire ants were performed in a greenhouse in rectangular plastic tubs (60 cm long by 30 cm wide by 30 cm high) containing soil and fire ant colonies. Plants were grown under ambient light and temperature conditions between April and September. This experiment was repeated twice with each repetition consisting of a total of eight tubs, four replicates containing fire ant colonies and four replicates without fire ants. Freshly excavated fire ant colonies, still in combination with the excavated soil, were placed in tubs previously coated with Fluon on the top 15 cm of the inside walls to prevent fire ant escape. Colony soil was mounded at one end of the tub, and pre-wet MetroMix350 (Scott-Sierra Horticultural Products Co., Marysville, OH) soil was added to bring the soil to a depth of 15 cm through out the tub. Ant colonies in all container studies were provided with six frozen crickets three times a week and had continual access to water in cotton stoppered test tubes for the

duration of the experiments. A total of 40 soybean seeds was planted at a depth of 2 cm in each tub. Seeds were 2.5 cm apart in three rows that were 2.5 cm apart and placed at the end of the tub opposite to the fire ant colony. Seedlings were observed and scored for number of emerged seedlings and amount and type of damage at 7 d after sowing.

**Soybean Plant Development in Association with Fire Ants.** The effect of fire ants on mature plant development was determined in experiments terminated 90 d after sowing. These experiments were performed as described for germination studies with the exception that only 12 seeds were planted per tub and thinned to six plants per tub 7 d after sowing by saving the healthiest appearing seedlings. Tubs were watered with a dilute fertilizer solution (2 g liter<sup>-1</sup> Peter's 20-20-20, Grace/Sierra Horticultural Products, Milpitas, CA) once weekly (1 liter) for the first 30 d and then three times a week (1 liter each time) for the duration of the experiment. After 90 d, the number of pods and the fresh weight and dry weight of leaves, stems, pods, and roots were determined. Dry weights were calculated by drying the plant material at 50°C until weight remained constant over time (3 d). The length of the longest root and number of root nodules were visually observed and recorded for each plant.

**Carbohydrate Analysis of Seedling and Mature Plant Tissues.** Comparison of the phagostimulant carbohydrate content of seedling and plant tissue was carried out to determine if differences in the levels of these sugars could explain the apparent difference in susceptibility of tissues to fire ant damage. Soybean seeds were germinated on a paper towel moistened with 0.01% aqueous sodium propionate (fungal inhibitor). The seedlings were sampled when the roots were 6 cm long. Roots, stems, and leaves were collected from field-grown mature soybean plants. Whole seedlings, roots, stems, and leaves were oven dried at 80°C to a constant weight. Each sample was ground in a mortar and pestle, weighed, and extracted with hot 60% aqueous ethanol. The mixture was heated in a boiling water bath for 10 min centrifuged to remove particulate matter. This process was repeated and the two extracts combined. The aqueous alcohol extract was vacuum evaporated until an aqueous solution remained. The aqueous solution was extracted three times with chloroform to remove contaminating lipids. These samples were subsequently used for carbohydrate analysis. An aliquot of each sample to be analyzed was evaporated and an equal volume of Tri-Sil Z (trimethylsilylimidazole in dry pyridine, 1.5 mEq, Pierce Chemical, Rockford, IL) was added. Each sample was heated at 70°C for 45 min with a vortex mixing treatment at 30 min. Carbohydrates were analyzed by gas chromatography (GC) on a Varian 3700 equipped with a flame ionization detector (Varian Associates, Palo Alto, CA) and a 1.8 m × 2-mm i.d. glass column packed with 3% OV-17 on 120/140 mesh Gas-Chrom (Applied Science Laboratories, State College Pennsylvania, PA). The GC oven temperature was programmed from 100 to 250°C at 10°C/

min. Sucrose, glucose, fructose, and trehalose were used as standards to identify GC peaks (Calbiochem and Sigma, St. Louis, MO). Quantitative data were obtained using a glucose external standard; thus, quantities are relative to the detector response for glucose. Peak identification was made by direct GC comparison with silylated carbohydrate standards. The GC data were analyzed using a Varian Vista 401 Data Processor.

**Statistical Analyses.** All statistical analyses were performed with SigmaStat (1997). Means of fire ant feeding response to soybean tissues and plant performance data were compared by Student *t*-test. When data were presented as a percent value, statistical analyses were performed on arcsine transformed values. Comparisons of total sugar concentration in different plant tissues were done using the Tukey multiple comparison analysis of variance (ANOVA) procedure. Linear regression analysis was performed on data representing the fire ant response to various concentrations of sucrose standard.

## Results

### Phagostimulant Effect of Germinating Soybeans.

Figure 1 shows the fire ant feeding response to soybean seeds/seedlings and the increase in fresh weight of the seedlings as they germinate. Foraging/feeding activity occurred only after the soybeans began to imbibe water. Dry seed did not induce a foraging/feeding response significantly greater than that induced by water ( $t = -2.201$ ,  $df = 8$ ,  $P = 0.059$ ); however, foraging activity on seed/seedling tissues from all other germination stages was significantly greater than that for water [G-1 ( $t = -9.128$ ,  $df = 8$ ,  $P < 0.001$ ); G-2 ( $t = -6.689$ ,  $df = 8$ ,  $P < 0.001$ ); G-3 ( $t = -9.476$ ,  $df = 8$ ,  $P < 0.001$ ); G-4 ( $t = -13.679$ ,  $df = 8$ ,  $P < 0.001$ ); G-5 ( $t = -4.820$ ,  $df = 8$ ,  $P = 0.001$ )]. After the G-1 stage, seedlings were developed enough that the response to the root and shoot/cotyledon portions could be compared. Although the overall response of the ants to the seedling tissues remained nearly constant among the G-1 to G-4 stages, over the time of the experiment there was a shift in foraging activity from the cotyledon/shoots to the roots. The foraging response to roots was significantly greater than that for the combined stem/cotyledon tissues at the G-2 ( $t = -2.838$ ,  $df = 8$ ,  $P = 0.022$ ), G-4 ( $t = 4.433$ ,  $df = 8$ ,  $P = 0.002$ ) and G-5 ( $t = 2.758$ ,  $df = 8$ ,  $P = 0.025$ ) stages, but not for the G-3 stage ( $t = 0.378$ ,  $df = 8$ ,  $P = 0.715$ ). Fire ant foraging response to five day-old seedlings (G-5) increased to an index value of >55% with >80% of that being associated with the roots, even though the roots comprised only 16% of the total seedling fresh weight. The shift of foraging activity toward the roots could not be explained by a selective increase in biomass of the roots over the cotyledon/shoots. Although the biomass increased for both tissue groups, the greatest increase was observed for the cotyledon/stems. It was clear that a preference for roots over shoots/cotyledons emerged as the seedlings developed.

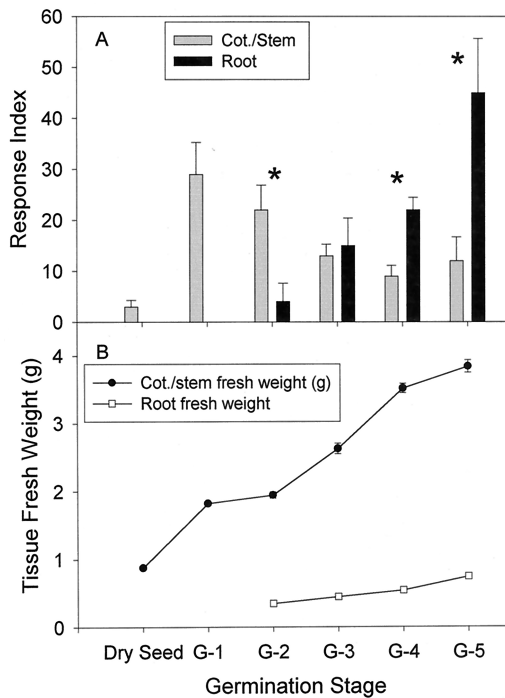


Fig. 1. Changes in fire ant phagostimulant response to soybean during germination and young seedling growth. (A) Mean  $\pm$  SE,  $n = 5$ , phagostimulant ranking versus soybean plant seed/seedling. Positive standard was 1.0% sucrose and the neutral standard was water (see *Methods and Materials* for phagostimulant bioassay details). \* Indicates the values for cotyledon/stem and root tissue within the indicated germination stage were significantly different from both the positive and neutral standards at  $P = <0.05$  level of probability. (B) Mean  $\pm$  SE,  $n = 20$ , fresh weight of seed/seedling tissues during seed development. G-1 through G-5 represent the number of days the seeds were allowed to germinate at 25°C in the dark and in rolled paper towels. At 2 d of germination the roots were distinct enough from the stem-cotyledon tissues to allow separate weight determinations.

**Effect of Fire Ant Association on Soybean Development.** Fire ant influence on soybean plant development was studied by germinating seeds planted in soil within containers, some of which housed fire ant colonies. Seedling damage was significantly higher with seedlings germinated in containers containing fire ants ( $t = -4.867$ ,  $df = 14$ ,  $P = <0.001$ ), Table 1. The most notable damage was to the cotyledons (Fig.

2A). More than 35% of the ant-associated seedlings had lesions on the cotyledons, whereas, only 2.3% of nonant associated plants had lesions. The fire ant feeding damage did not significantly affect seedling tissue development because there was no significant difference between the percent of malformed seedlings ( $t = -0.804$ ,  $df = 14$ ,  $P = 0.435$ ). Although the percent of seedling emergence was not significantly different between fire ant associated plants and control plants ( $t = 0.215$ ,  $df = 14$ ,  $P = 0.833$ ), the number of delayed emergence seedlings was significantly higher in fire ant associated plants ( $t = -2.164$ ,  $df = 14$ ,  $P = 0.048$ ). Emergence rate is often used in seed analysis to assess the quality of a given seed lot (AOSA 1983) and to assess seedling vigor. It is therefore apparent that fire ant association during soybean germination reduced the vigor of the developing seedlings.

Fire ant influence on plant development was most notable when plants were grown to the point of seed development in continual contact with the ants. Fire ant association significantly influenced root length ( $t = 6.147$ ,  $df = 6$ ,  $P = <0.001$ ) fresh weight ( $t = 7.417$ ,  $df = 6$ ,  $P = <0.001$ ) and dry weight ( $t = 3.813$ ,  $df = 6$ ,  $P = 0.009$ ), root nodule development ( $t = 8.550$ ,  $df = 6$ ,  $P = <0.001$ ), and pod fresh weight ( $t = -2.406$ ,  $df = 46$ ,  $P = 0.02$ ) and dry weight ( $t = -2.643$ ,  $df = 46$ ,  $P = 0.011$ ). There was a 39 and 28% reduction in root fresh and dry weights, respectively (Fig. 3). This was associated with a 32% reduction in the length of the root mass in fire ant associated plants as compared with control plants, Table 2. Nodule formation induced by the nitrogen fixing *Bradyrhizobium* bacteria was also inhibited in ant-associated plants with an 81% reduction in the number of nodules in comparison to the control plants (Table 2). The nodules that were visible on the ant-associated plants were shriveled and much smaller than those on control plants, suggesting that they were either non- or poorly functional (Fig. 2 B and C). Despite the reduction in root biomass and nodule formation, fresh and dry weights of pods produced per plant increased 24 and 43, respectively, when associated with fire ants (Fig. 3). These data suggest that some of the biomass produced by the plant was redirected to the developing pods and away from root/nodule development. However, when biomass data from all plant parts are combined, fire ant association still resulted in a 25% loss of total plant fresh weight and an 11% loss of total plant dry weight.

Table 1. Fire ant effect on soybean seed germination and plant growth

	% seedling emergence	7-d-old seedlings			90-d-old plant	
		% seedlings damaged	% of malformed seedlings <sup>a</sup>	% late emerging seedlings <sup>a</sup>	Length of roots*	No. root nodules
Ant associated plants	97.4 $\pm$ 1.0	35.9 $\pm$ 6.8*	11.8 $\pm$ 2.8	6.4 $\pm$ 2.1**	27.5 $\pm$ 1.9*	14.7 $\pm$ 1.6*
Control plants	97.4 $\pm$ 1.0	2.3 $\pm$ 0.9*	8.6 $\pm$ 2.9	1.6 $\pm$ 0.7**	40.7 $\pm$ 1.0*	77.7 $\pm$ 7.2*

\* Values are significantly different at  $P < 0.001$ . \*\* Values are significantly different at  $P < 0.05$ .

<sup>a</sup> Malformed and late emerging seeds were determined following the criteria indicated in AOSA (Rules for Testing Seeds) 1983.



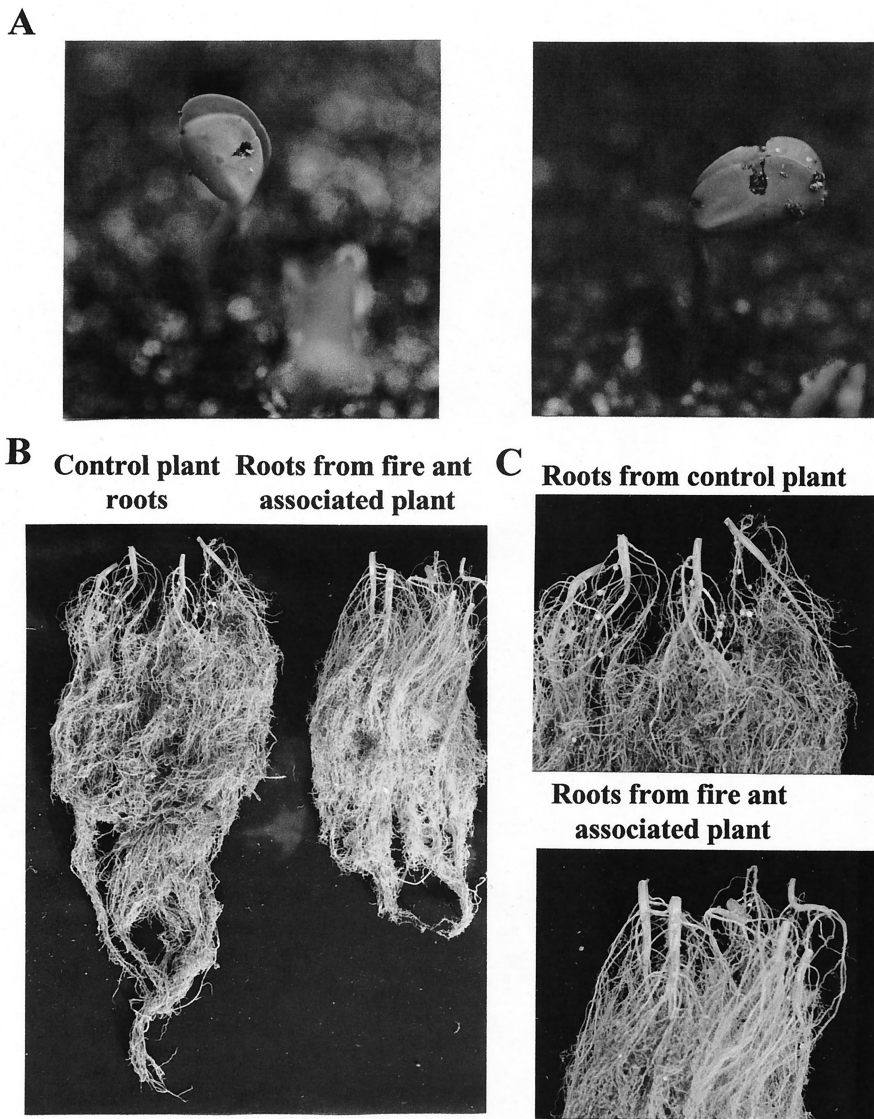


Fig. 2. Visual damage to soybean 7-d-old seedlings and roots from mature plants developed from seeds germinated in soil in the presence of fire ants. (A) Cotyledon damage observed on 7-d-old seedlings. (B) Comparison of soybean roots of 90-d-old plants grown in tubes containing soil either with or without a complete fire ant colony. (C) Close-up of roots shown in B shows differences in *Bradyrhizobium* induced root nodule formation between fire ant associated and control plants.

**Phagostimulant Carbohydrates Associated with Soybean Tissues.** Phagostimulant studies with seedlings showed a fire ant preference for roots over other seedling parts (Fig. 1). A similar preference was suggested with mature plants where root biomass and nodule formation was greatly reduced but no significant interaction with above-ground plant parts was ever observed. Soluble carbohydrates were quantified in plant tissues to determine if differences in the allocation of phagostimulant carbohydrates in different plant parts were associated with fire ant preferences. Measurable levels of fructose, glucose, and sucrose were found in all soybean tissue (Table 2); however, low levels of trehalose were detected only from seed-

lings. When the phagostimulant carbohydrates are taken as a group, the difference in the mean values among the treatment groups were significantly different ( $F = 4.868$ ,  $df = 3$ ,  $P = 0.01$ ). The germinating soybean seedlings contained higher mean amounts per gram of tissue than the mature plant parts, but only the roots and leaves were significantly different. The percent water in roots and leaves was not different; however, the stems had significantly less water. Because fire ant phagostimulant studies always compare fire ant response to water, where response to water is set as the zero response ranking, the higher level of water in the roots should not be the cause of the different response observed between roots and stems

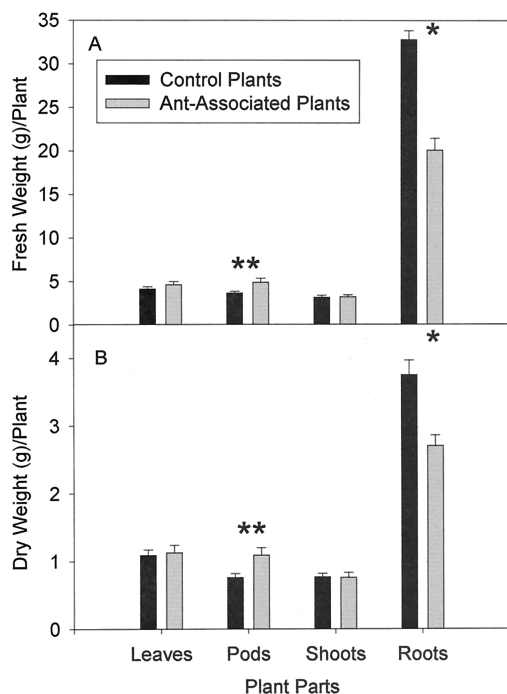


Fig. 3. Fire ant induced changes in 90-d-old soybean plant component weights. (A) Comparison of the mean  $\pm$  SE,  $n = 27$ , fresh weight of various plant parts from fire ant associated and control plants. (B) Comparison of the mean  $\pm$  SE,  $n = 27$ , of dry weights of various plant parts from fire ant associated and control plants. \* Indicates the results were significantly different at  $P = 0.01$  level of probability. \*\* Indicates a significant difference at  $P = 0.05$  level of probability.

(Fig. 1). Seedlings at the developmental stage tested contained significantly more water than the mature plant parts. Based on the mean values for the total carbohydrate and percent water, the potential carbohydrate concentration (wt./wt.) in aqueous solution in fresh tissue can be estimated. The percent phagostimulant carbohydrates in whole seedlings and mature plant stems, leaves, and roots were estimated to be 0.65, 0.35, 0.19, and 0.16%, respectively. Phagostim-

Table 2. The carbohydrate content of soybean seedlings and mature plant parts

Carbohydrate	Plant part			
	Roots	Stems	Leaves	Seedlings
Fructose	1.4 $\pm$ 2.0	6.7 $\pm$ 3.1	3.1 $\pm$ 2.7	6.4 $\pm$ 3.1
Glucose	0.7 $\pm$ 2.0	2.7 $\pm$ 1.7	0.5 $\pm$ 0.5	2.2 $\pm$ 1.0
Sucrose	1.4 $\pm$ 2.3	1.6 $\pm$ 1.0	0.7 $\pm$ 0.6	4.1 $\pm$ 1.9
Trehalose	ND	ND	ND	0.7 $\pm$ 0.4
Total	3.5 $\pm$ 5.4A	10.9 $\pm$ 5.7AB	4.4 $\pm$ 3.7A	13.4 $\pm$ 6.4B
% Water	69.3 $\pm$ 2.6A	62.8 $\pm$ 1.8B	70.4 $\pm$ 1.8A	79.1 $\pm$ 1.8C

Results are expressed as the mean  $\pm$  SD mg carbohydrate per gram of dry weight ( $n = 6$ ). Means for total carbohydrate or percent water followed by a different letter are significantly different from other means at  $P < 0.05$  level of probability as determined using the Tukey multiple comparison ANOVA. ND, Not detected.

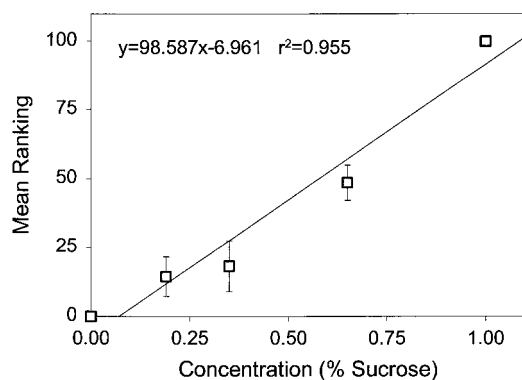


Fig. 4. Sucrose concentration versus the mean  $\pm$  SE,  $n = 8$ , ranking. Positive standard was 1.0% sucrose and the neutral was water (see *Methods and Materials* for phagostimulant bioassay details). Linear regression is plotted with the regression equation included on the graph.

ulant bioassays were performed using equivalent concentrations of sucrose (Fig. 4). Previous work showed that sucrose, glucose, and fructose had similar phagostimulant effects on fire ants (Vander Meer et al. 1995), allowing the comparison of the results from these three sugars to the sucrose standard. In comparisons where the fire ant response to water was set as the zero mean ranking, all tested sucrose concentrations were better phagostimulants than water, indicating that the total sugar concentrations in the soybean tissues should also be phagostimulatory.

## Discussion

Although it is well documented that the fire ant adversely affects the yield of soybeans, the nature of this yield reduction is poorly understood. Fire ant damage to seeds has been well characterized in a number of crops including soybean (Morrison et al. 1997); however, studies on fire ant interactions with crops throughout the growing season have been less thorough. It has been suggested that decreased yield is attributable to mound interference with harvesting and to fire ant damage to germinating seeds, resulting in fewer plants per row (Adams et al. 1983). Direct feeding on leaves, stems, or flowers of mature soybean plants has not been observed; however, results of  $^{32}\text{P}$  radioisotope studies indicate that the fire ant might feed on some part of the mature soybean plant (Smittle et al. 1983; Adams et al. 1983). Our data indicate that fire ants significantly reduce soybean root growth and inhibit nitrogen fixing nodule formation. Despite findings that phagostimulant levels of sugars are present in all seedling and mature plant tissues, and that sugars in mature soybean stems are higher than in the leaves or roots, there was no observation of fire ants feeding on any above-ground plant tissues. This work supports previous suggestions that fire ant feeding-damage activity occurs exclusively underground, e.g., young seedling tissue and roots. At first, this suggests that there may simply be

greater underground foraging activity compared with that above ground. However, our phagostimulant studies with seedling tissues performed in open trays, where fire ant workers had equal access to all seedling tissues, demonstrated a clear preference for foraging on roots as compared with stem or cotyledon tissues.

Of particular interest, was the time-dependent shift in fire ant activity from the stem/cotyledon to the roots (Fig. 1). It has been reported that as plant tissues green in response to light exposure, strong oxidizing compounds produced in the green tissues act as insect feeding deterrents (Duffy and Stout 1996). The germinating seedlings used in our phagostimulant studies were kept in the dark, and had not started to green; therefore, greening-induced oxidant formation is not a likely explanation. Alternatively, explanations that need to be tested include the following: either the roots are tenderer making them easier to feed on, or perhaps they produce fire ant attractants, phagostimulants, or aggregation stimulants that have a greater effect than factors produced by the stem/cotyledon.

Fire ants influenced the development of soybean plants beyond the seedling stages, affecting mature plant root and nodule development. It was previously reported that fire ant infestation reduced soybean yield 20–30% in field plots (Adams et al. 1983). The yield loss was attributed to a decrease in plant density in infested plots and not because of lower yields in individual plants. In fact, the number of pods per plant increased on fire ant-associated plants, which was explained as being a result of less crowding of the plants. Our greenhouse experiments also showed that soybeans grown in association with the fire ant yielded a greater number of pods per plant than those grown without ants. Thus, greenhouse experiments produce plant responses similar to those found under field conditions. Interestingly, the greenhouse results cannot be explained by plant density differences because densities in tubs were kept constant between ant associated and control tubs. Therefore, ant-stimulated increases in pod production per plant must result from factors other than changes in plant densities.

Although plants growing in association with ants had greatly altered root development, it cannot be determined when during plant development ant activity influenced the plant growth pattern. For example, it is conceivable that changes induced early on in seedling establishment may influence biomass partitioning between above and below ground growth. However, when our data are combined with previous work indicating uptake of radioactivity from  $^{32}\text{P}$ -fed soybean plants (Smittle et al. 1983), there is compelling evidence to indicate that fire ant activity with mature soybean plants is indeed occurring.

It is uncertain whether the loss in total soybean biomass resulted from reduced carbon fixation in fire ant-associated plants or from fire ants removing fixed carbon from the plant by direct feeding on the plant root system. Either way, fire ant association could limit per plant productivity in the field. In the previously reported field experiment, fire ant association did not lead to a per plant yield reduction; however, it is

important to note that the plants were continually irrigated and provided with adequate fertilizer. The major disadvantage to reduced root mass would be the limitation on water uptake. This was not a problem in our study or the discussed field studies because of irrigation. In production fields, soybeans are typically not irrigated, therefore yield losses during drought times may be even more severe because of fire ant infestations.

Our results show for the first time, the impact of fire ants on soybean root development, and indicate that feeding on the root system could be a major source of carbohydrate to support fire ant colony carbon requirements. There are two important implications from this work. First, this information provides direct proof that ants do interact with plant root systems; supporting the theory that a large portion of the carbohydrates obtained to support the colony comes directly from plant feeding (Smittle et al. 1983). Second, because fire ant damage reduces root biomass, we hypothesize that fire ant-associated plants in the field may experience yield-limiting water stress much earlier than a plant with a deeper root system.

This work also indicates the importance of studying the interaction between fire ants and plants to provide information on how fire ant energy requirements are supported. It is apparent that fire ant activity on plant roots is significant but has largely been overlooked because of the difficulty in observing the phenomenon of ant–root interactions. Understanding this process may be useful in designing strategies to reduce fire ant densities, or at least reduce losses in crop yields that result from fire ant infestations.

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